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Are you a fellow in training in an American Board of Obstetrics and Gynecology approved program leading to certification in Reproductive Endocrinology and Infertility? No
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Awards:
IRB Approval: No human subjects or human tissue was utilized in the studies described in the abstract.
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Accept Complete Responsibility: I accept complete responsibility for the data at the time of submission.

Title: COMPARISON OF POST-WARMING DEGENERATION AND APOPTOSIS OF PORCINE OVARIAN TISSUE FOLLOWING VITRIFICATION USING THE OHIO-CRYO DEVICE AND SLOW CRYOPRESERVATION
Objective: Vitrification is a promising alternative to slow freezing. Our objective was to compare vitrification of ovarian tissue using the Ohio-Cryo device with slow cryopreservation and examine the post-warming degenerative and apoptotic changes.

Design: In vitro study

Materials and Methods: 9 porcine ovaries were collected immediately post-mortem. After removal of medulla, ovarian cortex chopped into tiny pieces (1253 mm) and divided into 5 groups. Group I = fresh control; group II= spontaneously degenerate (4°C for 5 days); group III = induced cryodamage (direct plunging in LN₂); group IV = vitrification using Ohio-Cryo protocol and group V = slow cryopreservation. After warming, tissues were incubated for 2 h in culture media at 37°C and 5% CO₂ and fixed. For histological assessment paraffin embedded sections (5μm) were stained with H&E and scored as: 0 = no degenerative changes; 1 = slight degenerative changes and 2 = severe degenerative changes (modified Wood's criteria, 1998). Apoptosis was examined by colorimetric fragmentation detection kit and scored as 0: no color; 1 = mild color, 2= moderate & 3 = intense color.

Results: Mean ± SD of degenerative and apoptotic score in different groups are shown in

<table>
<thead>
<tr>
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<th>Fresh (Group 1)</th>
<th>Cryo-injured (Group 3)</th>
<th>Slow Freezing</th>
<th>Vitrification</th>
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</thead>
<tbody>
<tr>
<td><strong>Degenerative score</strong></td>
<td></td>
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<tr>
<td>Primordial</td>
<td>0.13 ± 0.15</td>
<td>0.63 ± 0.50 (p=0.06)</td>
<td>1.44 ± 0.71 (p = 0.016)</td>
<td>0.33 ± 0.42 (p=0.038)</td>
</tr>
<tr>
<td>Preanral</td>
<td>0.47 ± 0.37</td>
<td>0.86 ± 0.37 (p = 0.06)</td>
<td>1.45±0.42</td>
<td>0.45±0.24</td>
</tr>
<tr>
<td>Antral</td>
<td>0.25±0.38</td>
<td>0.93±0.25 (p=0.22)</td>
<td>1.30 ±0.71 p=NA</td>
<td>0.56 ± 0.72 (p=0.21)</td>
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<tr>
<td><strong>Apoptotic score</strong></td>
<td>0.25 ± 0.08</td>
<td>1.14 ± 0.45 (p=0.012)</td>
<td>1.36±0.51</td>
<td>0.39±0.18 (p=0.17)</td>
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</tbody>
</table>

There were no significant difference between vitrification and slow freezing in apoptosis (p = 0.66) or in degenerative scores for primordial, preantral and antral follicles (p = 0.28, 0.055, 0.63 respectively).

Conclusions: Compared with slow cryopreservation, vitrification showed superior results in all evaluated scores except for the degenerative score of antral follicles. This could be due to the limited sample size. Although slow cryopreservation remains a viable option for ovarian tissue freezing, ovarian tissue vitrification using the Ohio-Cryo method may be a promising alternative.

Support: None

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